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| **Chi-Square (X2) Tutorial and Practice**  Mrs. Krouse, AP Biology, 2015-2016  **When do I use a Chi square (X2) test?**   * The X2 test is a statistical test to compare observed results with expected results to determine if there is a statistically significant difference between them. The observed results are usually the data collected during an experiment. The expected results are the results you predict before starting the experiment. * The calculation generates a X2 value; the higher the value of X2, the greater the difference between the observed and the expected results or the two different sets of data.  1. **Example #1**   For example, let’s say that we are doing genetic testing on a population of elephants. The initial population has elephants that are all heterozygous (Aa) for the trait of trunk length. Trunk length in elephants is controlled by a single gene, where the dominant allele (A) codes for the long trunk phenotype and the recessive allele (a) codes for the short trunk phenotype. Researchers sampled 100 offspring from this initial population and found the following phenotype frequencies. These are the OBSERVED results.  **Observed Results**   |  |  | | --- | --- | | **Phenotype** | **Number of Offspring Observed with this Phenotype** | | Long Trunks | 80 | | Short Trunks | 20 |   We can use or knowledge of genetics to predict our expected results. If we know that all parents in the initial population are heterozygous for the trait of trunk length, we can predict offspring phenotype frequencies using a Punnett square (see below).   |  |  |  | | --- | --- | --- | |  | A | a | | A | AA (Long Trunk) | Aa (Long Trunk) | | a | Aa (Long Trunk) | aa (Short Trunk) |   According to this Punnett square, we would predict that 75% of the offspring would have long trunks and 25% of the offspring would have short trunks. If we know the number of offspring sampled (100), we can use this and the predicted phenotype frequencies to predict the number of offspring elephants out of the sample with long trunks and short trunks. These are the EXPECTED results.  **Expected Results**   |  |  |  | | --- | --- | --- | | **Phenotype** | **Predicted Frequencies** | **Number of Offspring Predicted with this Phenotype** | | Long Trunks | 75% or 0.75 | 100 x 0.75 = 75 | | Short Trunks | 25% or 0.25 | 100 x 0.25 = 25 |     Using the Chi square test, we would be able to determine if there is a statistically significant difference between the observed results (80 long trunks / 20 short trunks) and the expected results (75 long trunks / 25 short trunks)   1. **Example #2**   Suppose we were trying to determine if there is a statistically significant difference between the number of turtles with brown shells and the number of turtles with green shells in a population. We sampled 200 turtles from the population and found that 92 of them were brown-shelled and 108 of them were green-shelled.  For the purposes of our Chi square test, we will call these our OBSERVED results.  **Observed Results**   |  |  | | --- | --- | | **Turtle Shell Color** | **Number of Turtles** | | Brown Shells | 92 | | Green Shells | 108 |   If we had predicted that we would find equal numbers of brown-shelled turtles and green-shelled turtles (i.e. 50% of each), then our EXPECTED results are as follows…  **Expected Results**   |  |  |  | | --- | --- | --- | | **Turtle Shell Color** | **Predicted Frequencies** | **Number of Turtles** | | Brown Shells | 50% or 0.50 | 200 x 0.50 = 100 | | Green Shells | 50% or 0.50 | 200 x 0.50 = 100 |   Using the Chi square test, we would be able to determine if there is a statistically significant difference between the observed results (92 brown shells / 108 green shells) and the expected results (100 brown shells / 100 green shells).  **How do I perform a Chi square test?**   1. **State the null hypothesis**  * This is a negative statement, basically saying that there is no statistically significant difference between observed and expected results   For Example #1 given above, our null hypothesis would be… “There is no statistically significant difference between the number of long-trunked and short-trunked offspring observed in the population and the number of long-trunked and short-trunked offspring expected based on our Punnett square.”  For Example #2 given above, our null hypothesis would be… “There is no statistically significant difference between the number of brown-shelled and green-shelled turtles observed in the population and the number of brown-shelled and green-shelled turtles expected.” Because we expect to have equal numbers of each shell color, we could write this null hypothesis more simply as… “There is no statistically significant difference between the number of brown-shelled and green-shelled turtles in the population.”   1. **Determine your expected values**  * The way you calculate your expected values will be different for each situation * We have already done this for Example #1 and Example #2 given above. * Expected and observed values are always whole numbers. This is why we converted our expected frequencies (ex: 75% long trunks and 25% short trunks for Example #1) to whole numbers of elephants (ex: 75 long trunks and 25 short trunks.   **3. Calculate chi2**   * The formula is: * Where o = observed value, e = expected value, and ∑ = the sum of * So you would need to calculate separately for each value (ex: each phenotype from Example #1) and then add the results together. See a sample calculation below for Example #1.  |  |  |  |  | | --- | --- | --- | --- | | **Phenotype** | **Observed** | **Expected** | **(o – e)2/e** | | Long Trunks | 80 | 75 | (80-75)2/75 = 0.33 | | Short Trunks | 20 | 25 | (20-25)2/25 = 1 | |  |  | Total (this is your X2 value!) | 1.33 |   **4. You will also need to know the degrees of freedom.**   * This is calculated using the formula (n-1) * where n = the number of sets of results.(ex: the number of possible phenotypes from Example #1) * For Example #1… degrees of freedom = n-1 = 2-1 = 1   **5. Compare the X2 value against a table of critical values.**   * On the table below, refer to the row that corresponds to the correct number of degrees of freedom for your data set * Look up the critical number at the intersection of the correct degrees of freedom and the p = 0.05 column. “p” stands for probability level. Scientists almost always use a 0.05 probability level. * For Example #1, the critical value (aka critical number) is 3.84 (see circled value on the chart on the next page).     **6. Make a conclusion**   * If the X2 value that you calculated in Step 3 is higher than the critical value at the p = 0.05 level then you can reject the null hypothesis. In other words, there is a statistically significant difference between the observed and expected results. (i.e. the observed results do not match the expected results)   *Note: A high X2 value corresponds with a low p value (below 0.05)*   * If the X2 value is less than the critical number then you fail to reject (or support) the null hypothesis. In other words, there may not be a statistically significant difference between the observed and expected results. (i.e. the observed results may match the expected results, and any deviations from these expected results may be due to chance alone)   *Note: A low X2 value corresponds with a high p value (above 0.05)*  For Example #1, the calculated Chi square value (1.33) is lower than the critical value (3.84), so we fail to reject (or support) the null hypothesis. This means there may not be a statistically significant difference between the observed and expected results. In other words, there may not be a statistically significant difference between the number of offspring observed with each phenotype and the number expected to have each phenotype based on the Punnett square.  **\*\*\*Now… please complete the two problems on the following pages to practice using Chi square analysis\*\*\***  **Problem #1**  Naked mole rats are a burrowing rodent native to parts of East Africa[.](http://en.wikipedia.org/wiki/East_Africa) They have a complex social structure in which only one female (the queen) and one to three males reproduce, while the rest of the members of the colony function as workers. Mammal ecologists suspected that they had an unusual male to female ratio. They counted the numbers of each sex in one colony.   |  |  | | --- | --- | | **Sex** | **Number of animals** | | Female | 52 | | Male | 34 |   **State the Null hypothesis**  There is no statistically significant difference between the number of males and females.  **Calculate the expected results**  52 + 34 = 86 (total population size)  86/2 = 43 (number of males and females expected if there are an equal number of males and females)  **Calculate the chi-squared value**   |  |  |  |  | | --- | --- | --- | --- | | **Sex** | **Observed** | **Expected** | **(o – e)2/e** | | Female | 52 | 43 | 1.88 | | Male | 34 | 43 | 1.88 | |  |  | Total (this is your X2 value!) | 3.76 |   **What are the degrees of freedom?**  DF = 2 – 1 = 1  N = 2 (there are 2 sexes, so 2 sets of data)  **Compare the Chi square (X2) value with the critical value/number from the chart below**  **Make a conclusion** (Do you reject or fail to reject your null hypothesis? What does that mean for THIS scenario?)  Because the Chi square value (3.76) is lower than the critical value (3.84), we fail to reject (support) our null hypothesis. This means that there may not be a statistically significant difference between the number of males and females in the population. |
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**Problem #2**

You have been wandering about on a seashore and you have noticed that a small snail (the flat periwinkle) seems to live only on seaweeds of various kinds. You decide to investigate whether the animals prefer certain kinds of seaweed by counting numbers of animals on different species. You end up with the following data

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| **Type of Seaweed** | **Number of animals on each kind of seaweed** |
| serrated wrack | 45 |
| bladder wrack | 38 |
| egg wrack | 10 |
| spiral wrack | 5 |
| other algae | 2 |
| Total | 100 |

**State the Null hypothesis**

There is no statistically significant difference between the number of animals on each type of seaweed.

**Calculate the expected results**

100 (total number of animals) / 5 (number of types of seaweed) = 20 animals expected on each kind of seaweed if there are equal numbers of animals on each kind of seaweed

**Calculate the chi-squared value**

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| --- | --- | --- | --- |
| **Seaweed** | **Observed** | **Expected** | **(o – e)2/e** |
| serrated wrack | 45 | 20 | 31.25 |
| bladder wrack | 38 | 20 | 16.2 |
| egg wrack | 10 | 20 | 5 |
| spiral wrack | 5 | 20 | 11.25 |
| other algae | 2 | 20 | 16.2 |
|  |  | Total (this is your X2 value!) | 79.9 |

**What are the degrees of freedom?**

DF = 5 – 1 = 4

N = 5 (there are five types of seaweed, so 5 sets of data)

**Compare the calculated value with the critical value/number from the chart below**

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**Make a conclusion** (Do you reject or fail to reject your null hypothesis? What does that mean for THIS scenario?)

Because the Chi square value (79.9) is higher than the critical value (9.49), we reject our null hypothesis. There is a statistically significant difference between the number of animals on each type of seaweed.